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Laboratory Evaluation of the Pathogenicity of *Beauveria Bassiana* and *Metarhizium Anisopliae* to Larvae of the Banded Sunflower Moth, *Cochylis Hospes* (Lepidoptera: Cochylidae).

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LABORATORY EVALUATION OF THE PATHOGENICITY OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* TO LARVAE OF THE BANDED SUNFLOWER MOTH, *COCHYLIS HOSPES* (LEPIDOPTERA: COCHYLIDAE).

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ABSTRACT

Laboratory bioassays were conducted to assess the virulence of two entomopathogenic fungi, *Beauveria bassiana*, and *Metarhizium anisopliae* to 5th instars of the banded sunflower moth, *Cochylis hospes*,(Lepidoptera: Cochylidae). Temperature conditions of 20 and 25°C and high humidity, (near saturation) were nearly optimal for development of both fungi. Concentrations of 10^7 to 10^8 conidia/ml produced 100% mortality in 10 days or less and 10^6 conidia/ml produced 90% mortality at 21 to 26 days. Median lethal concentrations of conidia (LC_{50}) from *M. anisopliae* were 3.6×10^3 at 25°C and 4.1×10^3 at 20°C. The LC_{50} for *B. bassiana* was 14.9×10^4 conidia/ml at 20°C and 6.7×10^3 conidia/ml at 25°C. Although *B. bassiana* tended to be less virulent at 20°C, these differences were not significant. The high humidities required for germination and growth may reduce the usefulness of these fungi as control agents of *C. hospes* in the northern Great Plains. Further studies and field evaluations are needed to determine if there are microhabitats in the soil or on the sunflower head where the humidity is high enough for germination and growth of *B. bassiana* or *M. anisopliae*. Targeting of *C. hospes* stages in the soil to avoid contaminating the seed or oil with saprophytic fungal spores may be preferred to targeting the sunflower plant for reasons of preserving seed quality, marketing, and consumption.

Beauveria bassiana (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotinia: Hyphomycetes) are entomopathogenic fungi that occur in the soil, have a wide insect host range, and are distributed worldwide (Tanada and Kaya 1993). Virulence to insects varies with the fungal variety or strain, dose, host age, and environmental conditions, particularly temperature and humidity (Miller et al. 1983, Tanada and Kaya 1993). Infection usually takes place through chitinolytic penetration of the integument by fungal hyphae and both genera produce mycotoxins (Tanada and Kaya 1993). *Beauveria bassiana* and *M. anisopliae* are are being studied as alternatives to chemical pesticides for the control of a variety of insect species (Tanada and Kaya 1993, Miller et al. 1983) but neither has been significantly explored for microbial control of the banded sunflower moth *Cochylis hospes*, or other sunflower insect pests. The life cycle of *C. hospes* begins about the third week in July when eggs are laid on the bracts of sunflower. The larval

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stages feed on the florets and seeds of the sunflower head. The fifth and ultimate instar leaves the sunflower head in late August and early September to overwinter in the soil, and pupates in late spring (Westdal 1949, Charlet and Gross 1990, Charlet et. al. 1997, Beregovoy and Riemann 1989). The adults emerge about mid-July and begin a new generation. The objective of this study was to assess, in controlled laboratory conditions, the virulence of these fungi to fifth instars and pupae that overwinter in the soil. The latter stages were targeted for control in this study, because application to the sunflower head of large numbers of conidia from saprophytic fungi to control the earlier instars, could reduce the quality or acceptability of the seed or oil by consumers and the sunflower industry. The high humidity needed for fungal growth is more probable in the soil than on the sunflower capitulum where high humidity is incompatible with maintaining seed quality.

MATERIALS AND METHODS

Insects and rearing conditions. *Cochylis hospes* is routinely reared on insect diet in laboratory environmental chambers maintained at 28 ± 1°C, 50% relative humidity, and a 15:9 L:D cycle (Barker 1988). Fifth instars that had ceased feeding were selected primarily because this stage completes its development in the soil where it was suggested that control be targeted and, secondarily, it circumvented complications that would arise from diet spoilage in the presence of saprophytic fungi . In addition, ingestion of conidia was not required since infection of insects with fungi is primarily through the integument (Tanada and Kaya 1993). Cessation of feeding by the 5th-instar of *C. hospes* is marked by a change in color about 2 days after the molt to the fifth instar. The insect diet contains Benomyl (Bonide Products Inc. Yorkville, N.Y.) which will inhibit but will not prevent fungal growth, however, contamination of the diet is controlled primarily with the use of a sterile hood, materials, utensils, and control of excess moisture (Barker 1988).

Fungi. *M. anisopliae* (ATCC # 22099) was obtained from the American Type Culture Collection, Rockville, MD. A strain of *B. bassiana*, formulated into a commercial microbial insecticide Naturalis-L®, was obtained from Troy Biosciences, (Phoenix AZ). Naturalis-L and ATCC 22099 were stock preparations which were sub-cultured on potato dextose agar plates to produce conidia for bioassay.

Bioassay. Conidia from sub-cultures of Naturalis-L or ATCC 22099 stock were suspended in 10mM phosphoric acid buffered saline, pH = 7.0, prepared with 0.5 % Tween 80 to reduce clumping of the conidia and counted with a hemocytometer. Serial dilutions were prepared with the following concentrations of conidia/ml: 10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, or 0 (controls). This method of assay has a limitation in that it does not define precisely what dosage each larva received, but it does define the concentration of conidia to which each larva was exposed. A 1 and a 10µl sample of each concentration was streaked on potato dextrose agar plates to check for viability. Thirty, 5th-instar larvae were transferred to each of the tubes containing conidia and gently shaken for 5–10 sec. by inverting the tube. Longer periods of immersion were avoided because of detrimental effects on larval development and survival that were not related to infection. The 30 larvae were confined individually in 1 ml vented microfuge tubes with a piece of moist potting foam provided for pupation. Moisture was added to the potting foam and replenished as necessary to facilitate survival of pupae and their successful emergence as adults and to facilitate growth of the fungi. Tubes were vented by punching a hole (diameter 4mm) into the cap of the tube and covered with

Nitex (Tetko, Briarcliff Manor, NY) 2-ply polyester (70 threads per cm). The larvae were held at 20±1°C or 25±1°C in environmental chambers maintained on a 15:9 L:D cycle and 60–70% relative humidity. The humidity within the microfuge tubes, with the moist potting foam, was near saturation. Observations for mortality began on the second day after treatment and every other day thereafter for 21 or 26 d at 25 and 20°C, respectively, until the controls and treated insects that survived had pupated and emerged as adults. The LC_{50} of the treated larvae was determined at 21d (25°C) and 26 d (20°C). Cadavers that did not show obvious mycelial growth were macerated with a forceps and the debris streaked on potato dextrose agar and observed for mycelial growth. The bioassay was replicated eight times and an overall average response for each concentration was obtained by averaging the data from the eight replicates.

Statistical methods. POLO-PC (LeOra Software 1987, Berkeley, CA) was used to estimate the LC_{50}s and determine the Chi square goodness of fit. Jandel SigmaPlot (version 5, 1992 and version 2, 1995, San Rafael, CA) and Finney (1947) were used to plot probit regression equations.

RESULTS AND DISCUSSION

Mortality in relation to concentration of conidia and temperature. Mortality that resulted from treatment of *C. hospes* 5th instars with conidia from *B. bassiana* and *M. anisopliae* was 100% in 10 d or less after treatment with 10^8 and 10^7 conidia/ml at both temperatures. Fifth instars begin to pupate about 13 days after becoming fifth instars at 25°C (Barker 1994) but none of the fifths treated with 10^8 or 10^7 conidia survived to the pupal stage at 20 or 25°C. Mortality after treatment with 10^6 conidia of both fungi was approximately 90% at both temperatures over a period of 21 to 26 d (Table 1) while the controls and survivors completed development to the adult stage. The majority of fifths treated with 10^6 conidia per ml died as fifth instars because development of infected larvae to the pupal stage was retarded relative to the controls, although some pupated and then died. Diapause was not induced in the fifths held at 20°C in this study because the

Table 1. Mortality of *C. hospes* larvae after infection with conidia of *B. bassiana* or *M. anisopliae* at 20 and 25°C.

	[Conc]	20°C % mortality *26 d*	25°C % mortality *21 d*
M. anisopliae	10^6	95.8 ± 2.5	92.0 ± 8.5
	10^5	93.3 ± 1.9	83.3 ± 7.7
	10^4	68.0 ± 9.0	63.3 ± 18.4
	10^3	30.0 ± 8.7	37.5 ± 8.8
	10^2	11.1 ± 6.2	16.7 ± 10.9
	0	6.7 ± 2.2	3.3 ± 2.7
B. bassiana	10^6	88.7 ± 5.9	91.7 ± 3.3
	10^5	68.4 ± 8.4	73.3 ± 16.3
	10^4	48.9 ± 8.8	54.0 ± 12.7
	10^3	32.2 ± 5.1	35.3 ± 4.8
	10^2	10.7 ± 0.7	13.3 ± 6.9
	0	5.6 ± 4.3	1.3 ± 1.0

Table 2. Estimated median lethal concentrations of conidia for *C. hospes* 5th instars.

Treatment	Temp.	LC_{50} Conidia/ml	Lower limit 0.95	Upper limit 0.95	Slope ± S. E.
M. anisopliae	20°C	4,122	2,531	6,269	1.042 ± 0.114
M. anisopliae	25°C	3,651	2,111	6,184	0.662 ± 0.071
B. bassiana	20°C	14,956	8,122	26,389	0.621 ± 0.060
B. bassiana	25°C	8,160	5,268	12,486	0.592 ± 0.051

larvae were reared to the fifth instar under non-diapause inducing conditions (Barker 1994) and then placed at 20°C as control or test insects. Mortality trended lower for both fungi, at both temperatures, in response to exposure to lower concentrations of conidia. The estimated LC_{50} values of *M. anisopliae* conidia at 20 and 25°C were 4.1×10^3 and 3.6×10^3 conidia/ml at 26 d and 21 d, respectively (Table 2). The estimated LC_{50} values for larvae treated with *B. bassiana* at 20 and 25°C were 14.9×10^4 and 8.1×10^3 conidia/ml respectively (Table 2).

LC_{50} values and slopes in relation to temperature. Optimum temperatures for development, pathogenicity, and survival of fungi generally fall between 20 to 30°C (McCoy et al. 1988). The optimal growth temperatures for *Beauveria* are 23–25°C and 27–28°C for *Metarhizium* (Ferron 1981). Generally, there is a decline in virulence of entomopathogenic fungi with declining temperature (Deacon 1983, Rath et al. 1995) outside the optimal temperature range. The LC_{50} values at 20 and 25°C overlapped for both fungi indicating that the change of temperature from 25 to 20°C did not significantly affect mortality. The overlapping regression lines for *M. anisopliae* did not clearly demonstrate a trend toward lower virulence at 20°C. *B. bassiana* showed a trend toward reduced virulence at the lower temperature (Fig.1). The slopes of the regression lines for *B. bassiana* at 20°C and 25°C are not significantly different since the lines are nearly parallel but the lines reflect a lower virulence at 20°C than at 25°C. Chi-square heterogenity < 1 indicated a good fit of the regression equations to the data in each case and $P \leq 0.05$ in the Chi-square goodness of fit test.

The results of this study show that *B. bassiana* and *M. anisopliae* are pathogenic to *C. hospes* larvae in laboratory conditions where the temperature and humidity are controlled. The humidity level (around 90%) required by these fungi for optimal germination and infection (Deacon 1983) may limit their use as control agents to periods of high humidity. Studies of microhabitats in the sunflower head or the soil may indicate that in those environments the humidity levels are high enough for the development of these fungi. High humidity needed for fungal growth is more probable in the soil than on the sunflower capitulum where it is undesirable because it is incompatible with maintaining seed quality. Application to the sunflower head of large numbers of conidia from saprophytic fungi should be avoided since it could reduce the quality or acceptability of the seed or oil. Humidity levels in the soil are probably 90% under some climatic conditions, but targeting control to *C. hospes* stages in the soil can only be directed to the 5th instar which enters the soil to overwinter, or to the pupal stage which develops in the soil. Application of conidia to the soil in the fall or in the spring and early summer to control overwintering larval and pupal stages of the next generation of *C. hospes* is a possibility that circumvents objections to treatment of the sunflower capitulum. Infectivity of the fungus in the soil, however, could be slowed due to suboptimal temperatures that prevail in the fall and spring months. The total mortality from infection with these fungi may be unaf-

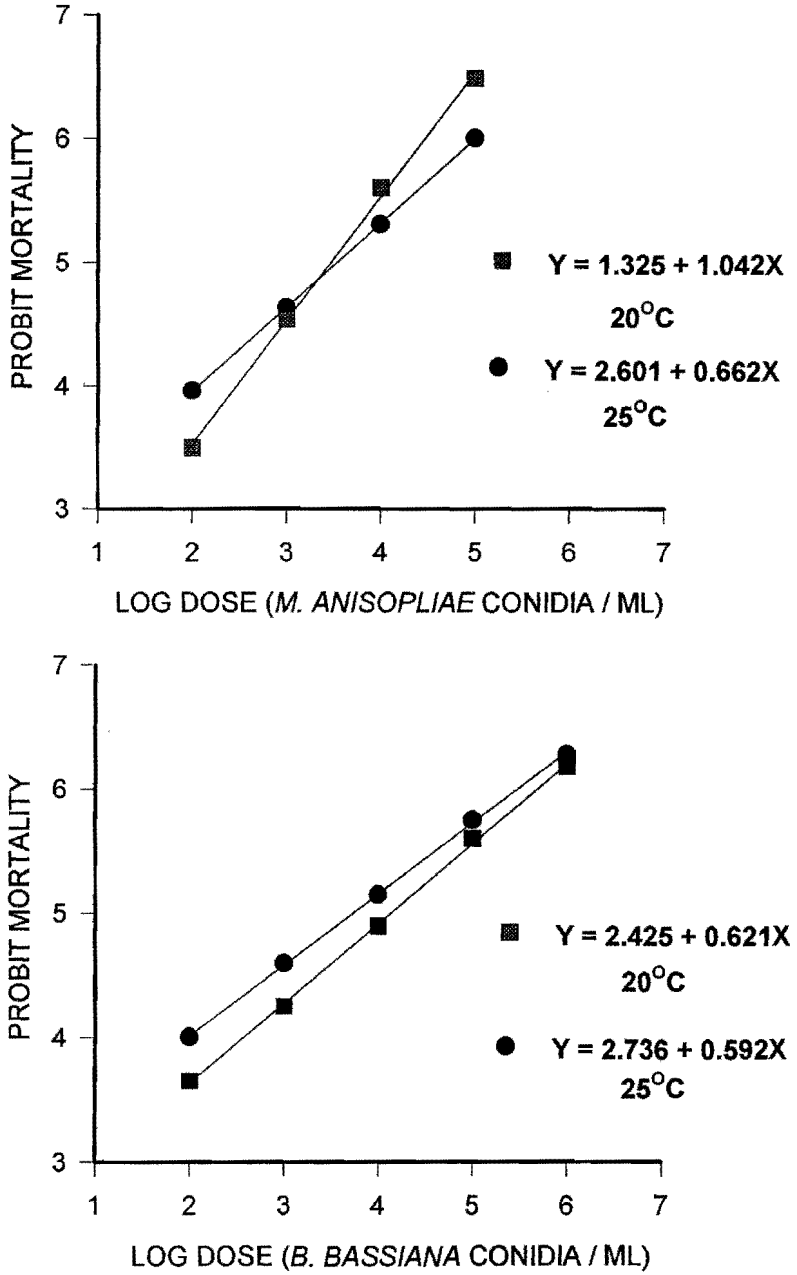

Figure 1. Probit regression lines for the mortality of *C. hospes* fifth instars treated with conidia from *M. anisopliae* and *B. bassiana* at 20 and 25°C.

fected at suboptimal temperatures except that it occurs over extended periods of time (Ferron 1978, Rath et al. 1995). Isolation or development of fungal strains that germinate and develop at lower temperature and humidity is another alternative.

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